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# Dechlorination Mechanisms of Chlorinated Olefins

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**The dechlorination of chlorinated hydrocarbons has been examined in detail. The reaction is catalyzed by cytochrome P-450 and occurs optimally in the presence of oxygen although some dechlorination may occur under anaerobic conditions. Halothane has been shown to undergo an oxidative dechlorination and a reductive defluorination. Enzymatic attack of chlorinated olefins and hydrocarbons is not on the carbon–halogen bond. Oxidative dechlorination of hydrocarbons is apparently initiated by an attack on the carbon atom and the halogen is then released from the oxidized carbon. The chlorinated olefins, on the other hand, are not easily dechlorinated enzymatically. The chlorines migrate readily across the double bond, therefore, cyclic chloronium ions must occur as intermediates. It is not clear at this time if epoxides are also intermediates in this conversion.**

Halogenated compounds when put in a biologic system are converted to a variety of products, which indicates that there is either more than one mechanism controlling the dehalogenation or there is more than one step in the dehalogenation reaction. The actual situation may represent a combination of these possibilities. There have been a number of mechanisms suggested to account for the reaction of the halogenated compounds known to occur. Reductive dehalogenation accounts for the conversion of carbon tetrachloride to chloroform (*1*) and chloroform to methylene chloride (*2*) as well as for the conversion of DDT to DDD (*3*). A transferase system has been described to account for the replacement of a halogen by glutathione with concomitant dehalogenation and subsequent mercapturic acid formation (*4*, *5*), Kubic et al. (*6*) have described the conversion of dichloromethane to carbon monoxide by microsomes which would indicate a partial oxidation reaction. Recently Ahmed et al. (*7*) reported a cytosol glutathione transferase which converted dihalomethanes to formaldehyde and inorganic halides. Work in our laboratory has yielded convincing evidence that chlorinated hydrocarbons are oxidatively dechlorinated by a microsomal enzyme system (*8*, *9*). It is becoming apparent that the mechanism of dehalogenation of chlorinated compounds is a function of the type of carbon skeleton to which the halogen is attached as well as the metabolic or physiologic conditions and therefore no single mechanism will apply for all classes of compounds.

Studies of enzymatic dehalogenation have largely centered on the saturated hydrocarbons and for that reason this report will begin with a discussion of the metabolism of a series of chlorinated ethanes and propanes. It should be emphasized that throughout this discussion there is a considerable amount of speculative thought although as much as possible the speculation will be separated from the actual facts.

A basic rule can be applied to enzymatic dehalogenation irregardless of whether it is oxidative or reductive, that is, there is no direct attack on the carbon–halogen bond, but the attack is on the carbon causing, secondarily the halogen release. Also, it should be remembered that when dealing with mechanisms of this type one should not necessarily think that what applies to one halogenated compound applies to all of similar structure.

Our initial studies on this subject began with a series of chlorinated ethanes and propanes to determine the relative rates of dechlorination and the effects of various amounts of halogenation on these rates. Table 1 shows the results of the studies of the chlorinated ethanes. It is apparent from this study that the optimum configuration for dechlorination is a dichloromethyl group. Hexachloroethane shows a considerable amount of dehalogenation although it was subsequently found that the hexachloroethane is

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unstable in an aqueous system and the amount of dehalogenation shown in Table 1 represents nonenzymatic dechlorination. As a follow-up to this study, Lowe et al. (*10*), utilizing molecular orbital calculations of this series of compounds, observed that the dihalomethyl carbon was the most positive, since it lacked an electron in one of the orbitals, thus making it an excellent target for nucleophilic attack. The monochloromethyl carbons undergo oxidation to a lesser extent while the trihalomethyl carbon cannot undergo an enzymatic oxidation. This all helps to substantiate the fact that the enzymatic attack is on the carbon rather than on the carbon-halogen bond. A second series of halogenated ethanes was examined, and the results are listed in Table 2. The ethanes were substituted with both chlorine and fluorine, in an effort to examine the effects of other halogens on the release of chlorine. The results were the same as those found with the chlorinated ethanes, that is, a dihalomethyl group is optimum for dehalogenation and that the presence of fluorine does not interfere with the dechlorination, either if it is on the same carbon as the chlorine or on an adjacent carbon.

**Table 1. *In vitro* dechlorination of chlorinated hydrocarbons by rat liver microsomes.**

| Substrate | Structure | Dechlorination, % |
|---|---|---|
| Chloroethane | $CH_3CH_2Cl$ | <0.5 |
| 1,1-Dichloroethane | $CH_3CHCl_2$ | 13.5 |
| 1,1,1-Trichloroethane | $CH_3CCl_3$ | <0.5 |
| 1,2-Dichloroethane | $CH_2ClCH_2Cl$ | <0.5 |
| 1,1,2-Trichloroethane | $CH_2ClCHCL_2$ | 9.8 |
| 1,1,1,2-Tetrachloroethane | $CH_2ClCCl_3$ | 0.8 |
| 1,1,2,2-Tetrachloroethane | $CH_2Cl_2CHCl_2$ | 6.0 |
| 1,1,1,2,2-Pentachloroethane | $CHCl_2CCl_3$ | 1.7 |
| Hexachloroethane | $CCl_3CCl_3$ | 3.9[a] |

[a]Nonenzymatic.

**Table 2. Dechlorination of labeled substrates.[a]**

| Substrate | Dechlorination, % |
|---|---|
| 1-Chloro-1-fluoroethane | 5.0 |
| 1,1-Difluoro-1,2-dichloroethane | 1.2 |
| 1,1,2,2-Tetrachloro-1,2-difluoroethane | <0.5 |
| 1-Chloro-1,1-difluoroethane | 0.6 |
| 1,1-Dichloro-1-fluoroethane | 1.0 |

[a]All substrates randomly labeled with $^{36}Cl$. Incubation carried out with rat hepatic microsomes as previously described (*8*).

There has been a considerable amount of interest in the biotransformation of the volatile anesthetics, a group of drugs which have been very useful in studies of dehalogenation. In Table 3 are shown the structures of the currently used agents. As can be seen, all but one contain the dihalomethyl group which was previously indicated to be optimal for enzymatic attack. In the case of halothane the attack is on the chlorobromomethyl group with the trifluoromethyl group being resistant to oxidative attack, thus the major product of the oxidation of halothane is trifluoroacetic acid (*11*). Methoxyflurane is also an excellent substrate for oxidative attack. This substrate can either be attacked at the ether linkage or at the dichloromethyl group. It is interesting that the enzyme, when given a choice between these two sites of attack, prefers to dechlorinate rather than cleave the ether since the dechlorination occurs at twice the extent that the ether cleavage occurs (*12*). When ether cleavage occurs, inorganic fluoride is released. Enflurane also contains the dihalomethyl group, but in contrast to the previous two agents this compound undergoes a very limited amount of dehalogenation (*13*). The ether group is probably not attacked since it is reasonably well-balanced by the fact that there are two fluorines on either side. Thus while it has not been clearly established, the chlorofluoromethyl group is probably the only site of attack. Isoflurane undergoes a very limited metabolism (*14*), and in this case the metabolism probably only occurs by way of ether cleavage, since the ether is not well balanced as was the case with enflurane, thus allowing a limited enzymatic attack.

**Table 3. Volatile anesthetics.**

| | Structure |
|---|---|
| Halothane | $CF_3CClBrH$ |
| Methoxyflurane | $CH_3OCF_2CCl_2H$ |
| Enflurane | $CF_2HOCF_2CClFH$ |
| Isoflurane | $CF_2HOCClHCF_3$ |

Equations (1) and (2) summarize what is currently believed to be the route of metabolism of halothane. Halothane undergoes an oxidative as well as reductive metabolism (*15, 16*). In the presence of oxygen halothane is oxidized to trifluoroacetic acid and it is believed, although not definitely proven, that the oxidation is by way of trifluoroacetaldehyde. As one decreases the oxygen it is noted that the halothane still undergoes metabolism, but in the absence of oxygen the pathway is considerably altered. There are two pieces of evidence to suggest that reductive metabolism of the halothane

$$CF_3CClBrH \xrightarrow{O_2} [CF_3CHO] \xrightarrow{O_2} \underset{\text{binds to proteins}}{CF_3COOH} \quad (1)$$

$$\begin{matrix}[CF_3CClBr] \longrightarrow CF_2{=}CClBr \searrow \\ [CF_3CClH] \longrightarrow CF_2{=}CClH \nearrow \end{matrix} \text{ binds to phospholipids} \quad (2)$$

molecule does occur: (1) inorganic fluoride is released enzymatically in the absence of oxygen; and

(2) there is evidence that the halothane molecule is activated to a highly reactive intermediate which binds to microsomal phospholipids and proteins. The binding to microsomal phospholipids does not occur in oxygen but increases as oxygen tension is decreased, while the binding to protein occurs under aerobic conditions although decreases slightly in low oxygen. It has, therefore, been proposed that possibly two intermediates are responsible for the binding to the phospholipids [Eq. (2)]. One is a reactive radical produced by the release of either the hydrogen or the bromine and/or there is a possibility that the radical can rearrange and cause the release of an inorganic fluoride resulting in a haloethylene which will also bind to phospholipids. Additional support for this possibility is derived from the fact that both $^{36}Cl$-labeled halothane and $^{14}C$-labeled halothane are activated to intermediates which bind to phospholipids while no $^{36}Cl$ labeling of protein is found suggesting that the intermediate binding to protein has lost the chlorine while the intermediate bound to phospholipids has not. It should be pointed out that if the olefinic compound binds to phospholipids, it does so in the absence of oxygen, thus suggesting that many olefinic compounds should be looked at in this manner and considered to be reactive enough to bind without undergoing either oxidation or reduction. One additional comment needs to be made concerning the reason for proposing two intermediates in the reductive pathway. If one compares the molar ratio of inorganic fluoride to amount of material bound to phospholipids, the inorganic fluoride, assuming that one fluoride per halothane molecule is released, accounts for only half of the total amount of halothane which is found bound to phospholipids. Thus we are assuming that some intermediate other than the olefinic compound is binding, thereby raising the possibility that it is the radical. To further strengthen the possibility that a reaction occurs as proposed in the absence of oxygen a comparison can be made with certain reactions of halogenated compounds which occur nonenzymatically. Eqs. (3)-(6) are examples of some reactions of a haloethane very much like halothane which undergoes a hydrogen extraction in the presence of basic methanol (*17*). The resulting intermediate spontaneously rearranges resulting in the loss of fluorine and the formation of an olefin. This olefinic compound then undergoes a nucleophilic attack and binds with the methanol ion. Therefore this reaction is very much like that which is proposed for the enzymatic reduction of halothane.

$$CF_3CHCl_2 + MeO^- \rightleftharpoons CF_3CCl_2^- + MeOH \quad (3)$$

$$CF_3CCl_2^- \longrightarrow Cl^- + CF_2{=}CCl_2 \quad (4)$$

$$CF_2{=}CCl_2 + MeO^- \longrightarrow MeOCF_2CCl_2^- \quad (5)$$

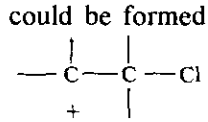
$$MeOCF_2CCl_2^- + MeOH \rightleftharpoons MeOCF_2CHCl_2 + MeO^- \quad (6)$$

Up to this point little has been said concerning the enzyme system responsible for dehalogenation. Both the oxidative and the reductive dehalogenations are mediated by cytochrome P-450. Table 4 shows the results of a study with the use of rat hepatic microsomes and 1,1,2-trichloroethane as the substrate. Similar results have been obtained by using a partially purified cytochrome P-450 system (*18*). The dechlorination is maximal in the presence of oxygen and requires NADPH; however, an important point to observe in these data is that under a nitrogen atmosphere the reaction does not stop. It decreases to approximately one-third to one-half of the rate which occurs under aerobic conditions, suggesting that 1,1,2-trichloroethane may undergo a reductive dehalogenation as well as an oxidative dehalogenation suggesting that even the saturated halogenated hydrocarbons may pass through an olefinic intermediate to the final product.

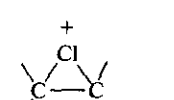
**Table 4.Dechlorination of 1,1,2-trichloroethane.**

| Additions to microsomes | Relative dechlorination | |
|---|---|---|
| | $O_2$ atmosphere | $N_2$ atmosphere |
| None | 7 ± 2 | 6 ± 1 |
| S | 4 ± 2 | 8 ± 3 |
| NADPH | 100 | 58 ± 7 |

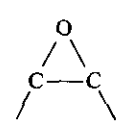
Turning now to the olefins, some insights to the way in which they are metabolized can be gained by examining the possible intermediate metabolites: (1) the carbonium ion; (2) the chloronium ion; and (3) epoxide. It is also possible that any one of the three intermediates could be formed and could exist at any given time. However, when talking about the enzymatic dechlorination of a chlorinated olefin, it



Carbonium ion

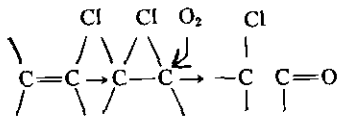


Cyclic chloronium ion



Epoxide

is important to remember that the same rule applies to the olefins as applies to the saturated hydrocarbons, that is, the chlorine is going to be released when the carbon containing the chlorine is oxidized. The studies with trichloroethylene are important in considering how olefins are metabolized in biological systems. In the first place, Daniel (*19*) has shown that trichloroethylene does not lose any chlorines when metabolized to trichloroacetic acid. Leibman (*20*) has proposed that there is an epoxide intermediate which rearranges to form a chloronium ion, a possibility also raised by Henschler (*21*). By proposing a chloronium intermediate it is possible to envision how the chlorines are conserved and trichloroacetic acid can be the major metabolite product. It also raises the possibility that chlorinated olefins will be enzymatically dechlorinated only if there can be no migration of chlorine to the adjacent carbon. Thus one might expect little or no dechlorination of all chlorinated ethylenes except perchloroethylene. On the other hand, the oxidative attack of chlorinated olefins does raise the possibility that a number of reactive intermediates might be generated and resulting in the possibility that these intermediates can bond to cellular constituents. Equation (7) points this out: the oxidative attack, when it occurs, forces any chlorine bound to the same carbon to migrate to the adjacent carbon if possible. If it is not possible, then the chlorine




(7)

bonded to the carbon which is oxidatively attacked is released. Thus the case of 1,2-dichloroethylene, as pointed out by Leibman (*22*), results in the appearance of dichloroacetic acid as the major product, a similar reaction to that occurring in the case of trichloroethylene is converted to trichloroacetic acid. In both cases a migration of the chlorine has occurred suggesting that a chloronium intermediate is produced. Whether or not an epoxide is necessary intermediate for the chloronium ion to be formed is not known at this time. However, it is perfectly possible that the epoxides can form in the case of 1,1-dichloroethylene or in the case of vinyl chloride, but one would have to question whether they would form in the case of the trichloroethylene or the 1,2-dichloroethylene.

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